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Treatment With Dimethyl Fumarate Attenuates Calcineurin Inhibitor-induced Nephrotoxicity

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Background. Cyclosporine A (CsA) is an immunosuppressive drug which has been widely used to prevent rejection after organ transplantation. However, its therapeutic use is limited by nephrotoxicity, in part mediated by oxidative stress. The present study aims to investigate the protective effects of dimethyl fumarate (DMF) on CsA-induced nephrotoxicity by enhancing the antioxidant defense system. **Methods.** Male Sprague–Dawley rats were treated with CsA (n = 8, 20 mg/kg per day intraperitoneally) or CsA + DMF (n = 7, 50 mg/kg per day orally) for 28 days. Renal function, histopathology, malondialdehyde (MDA), myeloperoxidase levels, and antioxidant enzyme expression were determined. **Results.** The DMF cotreatment ameliorated CsA-induced renal dysfunction as evidenced by significant decrease in serum creatinine (CsA 0.79 ± 0.02 mg/dL vs CsA + DMF 0.62 ± 0.04 mg/dL, $P = 0.001$) and urea (CsA 66.9 ± 0.4 mg/dL vs CsA + DMF 53.3 ± 2.6 mg/dL, $P < 0.0001$) levels, as well as improvement of creatinine clearance. Dimethyl fumarate also significantly decreased serum MDA and renal tissue MDA and myeloperoxidase contents. The protein expression of NAD(P)H quinone oxidoreductase-1, a major cellular antioxidant and detoxifying enzyme, was significantly enhanced by DMF administration in kidney. **Conclusions.** Administration of DMF has a protective potential against CsA nephrotoxicity. The protection afforded by DMF is mediated in part through inhibiting oxidative stress and inflammation and enhancing the antioxidant capacity.

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Cyclosporine A (CsA) is an important immunosuppressive drug that has been widely used for organ transplantation and for treatment of autoimmune diseases.¹ Immunosuppressive therapy with CsA is often limited by serious nephrotoxicity.² Renal dysfunction occurs in up to 30% of the patients.³ The mechanisms by which CsA causes acute reversible and chronic irreversible nephrotoxicity are not well understood but are thought to be, in part, due to oxidative stress and depressed endothelium-derived nitric oxide (NO) production.⁴ An increased production of free radical species and lipid peroxidation products has been demonstrated in kidney tissue under CsA treatment.⁵ The involvement of

oxidative stress was further supported by the finding that many antioxidants and free radical scavengers were capable of reducing experimental renal injury caused by CsA.^{6,7}

Dimethyl fumarate (DMF) was recently approved by the Food and Drug Administration for use in the treatment of patients with multiple sclerosis (MS). Although the mechanism of action of DMF is not clearly understood, DMF has been shown to inhibit proinflammatory cytokine production and nuclear factor (NF)- κ B signaling via inhibition of its nuclear translocation.⁸ Dimethyl fumarate carries, as well, a unique antioxidant profile.^{9–14}

In the present study, we sought to investigate whether DMF would have a protective effect against CsA nephrotoxicity.

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C.T., N.D.V., C.F.III, M.J.S., and H.I. participated in research design. C.T., N.D.V., L.R., and H.I. participated in the writing of the paper. C.T., S.L., L.R., K.V., M.T., C.P., S.L., and S.H.F. participated in the performance of the research. N.D.V., S.L., and H.I. contributed new reagents or analytic tools. C.T., S.L., L.R., K.V., M.T., C.P., S.H.F., and H.I. participated in data analysis.

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MATERIALS AND METHODS

Animals

Sixteen pathogen-free Sprague–Dawley male rats, weighing 230 to 250 g were purchased from Charles River (Wilmington, MA). The rats were housed under standard conditions (room temperature, 22°C; humidity, 50% \pm 5%; 12:12 hour light/dark cycle). The study was approved by Institutional Animal Care and Use Committee of University of California, Irvine.

Experimental Design

Sixteen rats were randomly divided into 3 experimental groups: (1) Control: given a vehicle. (2) CsA group (n = 8): were treated with CsA (Novartis Pharma AG, Switzerland) intraperitoneally at a dose of 20 mg/kg for 28 days. This dose was chosen based on earlier reports, which have been shown to successfully and consistently produce nephrotoxicity^{15,16}

(3) CsA + DMF group (n = 7): In addition to CsA, DMF (25 mg/kg, twice per day) was orally administered via gavage for 28 days. Experimental rats were given oral DMF (25 mg/kg, twice per day) dissolved in methyl cellulose (Sigma, St. Louis, MO). After the last dose of CsA, rats were kept individually in metabolic cages for 24 hours with free access to food and water to collect urine for the estimation of renal function. Blood samples were taken at the end of urine collection by cardiac puncture under anesthesia. The kidneys were immediately removed for evaluation.

Histopathological Analysis

Hematoxylin and eosin and periodic acid-Schiff staining was performed on, kidney tissue samples fixed in 10% buffered formaldehyde and embedded in paraffin. Evaluation of kidney histology was carried out in a blinded manner after a semiquantitative scoring system by 2 pathologists. Histologies were graded in regards to their tubular epithelial aspects, glomerular and vascular alterations according to modified Banff classification criteria.¹⁷ Each kidney sample had 20 randomly selected, nonoverlapping fields analyzed for hematoxylin and eosin and periodic acid-Schiff stains. Tubular injury (TI) was graded (0 to 3) based on the presence of tubular atrophy (=interstitial widening) and presence/degree of isometric tubular vacuolization: 0, no changes present; grade 1, $\leq 25\%$; grade 2, 26% to 50%; and grade 3, $>50\%$ TI involvement. Interstitial fibrosis (IF) was scored as a sign of architectural destruction: 0, no changes present; grade 1, 25%; grade 2, 26% to 50%; and grade 3, $>50\%$ TI involvement. Glomerular injury (GI) was graded 0 to -3 for sclerosis and mesangial matrix expansion as a marker for glomerular ischemia and damage. Renal arterioli were evaluated with respect to the presence of hyalinosis or sclerosis. Grade 0, no arteriolar changes; mild-moderate (grade 1), 1 arteriole affected; moderate-severe (grade 2), 1 to 2 arterioles affected; severe (grade 3), more than 2 arterioles affected.

Assessment of Renal Function

Blood and urine samples were analyzed for urea and creatinine using a commercially available kit (BioAssay Systems, Hayward, CA). Creatinine clearance was calculated using standard formula.

Measurement of Serum and Renal Malondialdehyde

Serum and renal tissue malondialdehyde (MDA) was measured using the TBARS assay kit (Cayman, Ann Arbor, MI) according to the manufacturer's instructions.

Myeloperoxidase Assay

The presence of myeloperoxidase (MPO) was used as an index of neutrophil accumulation in the kidney using the MPO colorimetric Assay kit (BioVision, Milpitas, CA) according to the manufacturer's instructions.

Protein Extraction and Western Blots

All steps for protein isolation were conducted at 4°C. Kidney samples were homogenized in tissue protein extraction reagent (RIPA, Thermo Scientific, NJ) with protease cocktail inhibitor (Sigma, St. Louis, MO). The resulting homogenate was centrifuged at $1600 \times g$ for 10 minutes. The supernatant total protein content was estimated using by the Bio Rad

Assay kit (Bio-Rad, CA). Equal 50 μg protein amounts were loaded into 4% to 12% 2-[Bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)-1,3-propanediol (Life Technologies, CA) and transferred to nitrocellulose membrane (GE Healthcare Biosciences, MA), which had been blocked in 5% nonfat dry milk in Tris-buffered saline with Tween (TBST) for 90 minutes. The membranes were then probed with rabbit polyclonal antibody to inducible nitric oxide synthase (iNOS) (1:400; Santa Cruz, CA), endothelial nitric oxide synthase (eNOS) (1:400, Santa Cruz, CA), glutamate-cysteine ligase catalytic (GCLC) (1:1000; Abcam, CA), superoxide dismutase (1:200; Santa Cruz, CA), heme oxygenase-1 (HO-1) (1:1000; Abcam, CA), and glyceraldehyde 3-phosphate dehydrogenase (1:5000; Cell Signaling, MA). Rabbit monoclonal antibody to catalase (1:5000; Rockland Immunochemicals, PA) and mouse monoclonal antibody to NAD(P)H quinone oxidoreductase-1 (NQO-1) (1:1000; Abcam) followed by secondary antirabbit or mouse immunoglobulin G (1:3000; Cell Signaling, MA) were also accessed. The membranes were developed with an enhanced chemiluminescence detection kit (Bio-Rad, CA) and exposed to X-ray film (Kodak, Rochester, NY). Glyceraldehyde 3-phosphate dehydrogenase was the internal reference for quantification. Immunoblots scanned by the densitometer were subjected to grey analysis with Image Quant TL 7.0 (GE Healthcare Life Sciences, PA).

Western Blot for Nuclear Nuclear Factor Erythroid2-Related Factor1

Dimethyl fumarate dissolved in methyl cellulose was orally administered via gavage for twice per day. Sprague-Dawley rats were treated with vehicle, 15 mg/kg or 30 mg/kg. One hundred milligrams of kidney cortex was homogenized on ice in lysis buffer containing 10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCL, 1 mM DTT and Protease Inhibitor Cocktail (Sigma-Aldrich, St. Louis, MO). Cellytic NuCLEAR Extraction Kit (Sigma-Aldrich) was used for isolation of nuclear proteins. Protein concentration in the tissue homogenates was determined by DC protein assay kit (Bio-Rad, US) and 100 μg of protein per sample was fractionated on 4% to 12% Novex Tris-Glycine gel (Invitrogen, Carlsbad, CA) at 120 V for 2 hours and transferred to nitrocellulose membrane (Invitrogen). The membrane was incubated for 1 hour in blocking buffer (1 \times TBS, 0.05% Tween-20 and 5% nonfat dry milk) and then overnight in the same buffer containing the given primary antibody. The membrane was washed 3 times for 10 minutes in 1 \times TBST before a 2-hour incubation in a buffer (1 \times TBST) containing horseradish peroxidase-conjugated anti-rabbit (1:3,000) (Abcam), anti-mouse (1:2000) (GE Healthcare), and anti-sheep (1:5000) immunoglobulin G (EMD, Millipore) secondary antibodies. The membrane was washed 3 times, then visualized with ECL prime Western blotting detection reagent (GE Healthcare) and developed by autoluminography. Band densities were quantified using the free Image J software (version 10.2) from the National Institutes of Health (www.imagej.nih.gov/ij/).

Statistical Analyses

All the results are presented as mean \pm standard deviation. Comparisons between the two were performed with Student *t* test, Mann-Whitney *U* test, as appropriate as appropriate using Stat View-J 5.0 software (SAS, Cary, NC). Statistical significance was defined as *P* value less than 0.05.

RESULTS

Effects of CsA and DMF on Body Weight Change and Urine Output

The CsA-treated group had a nonsignificant weight loss compared to the control group ($P = 0.12$). However, CsA + DMF group had significantly lower body weight increase compared to the control group ($P = 0.003$). Urine output was significantly increased in the CsA group ($P = 0.03$ vs. normal) and decreased by DMF cotreatment ($P = 0.01$ vs. CsA group). There was no significant difference between the control group and CsA + DMF group ($P = 0.21$). The similar tendency was observed in urine output divided by body weight (Figure 1A-C).

Effects of CsA and DMF on Renal Function

The effects of CsA and DMF on renal function are shown in Figure 1D to F. Serum creatinine (Cr) and urea were significantly increased ($P < 0.001$, $P < 0.0001$, respectively), and creatinine clearance was significantly reduced in the CsA-treated group. The DMF treatment significantly reduced serum Cr ($P = 0.001$) and urea ($P < 0.0001$) concentrations and significantly improved creatinine clearance in DMF + CsA group compared to CsA group ($P = 0.01$).

Histopathological Changes

The photomicrographs of kidney in experimental groups are depicted in Figure 2, and the histopathological scores are summarized in Figure 3. There is no histological change by DMF administration without CsA. Histological injury including tubular cell vacuolization, tubular atrophy, IF, arteriolar hyalinosis and glomerular mesangial matrix expansion were observed in CsA group (Figure 2E-H). These structural alterations were attenuated by DMF administration (Figure 2I-L).

There was a significant difference between the control and CsA groups in tubular atrophy, IF, GI, and arteriolar hyalinosis (Figure 3A-D). Tubular injury score was significantly reduced by DMF administration (Figure 3A; $P = 0.02$ vs CsA group). In addition, insignificant trends for improvements in GI and arteriolar hyalinosis scores were observed in the DMF group (Figure 3B and C; $P = 0.12$, $P = 0.10$, respectively vs CsA group).

Effects of CsA and DMF on Serum and Renal MDA Levels

As shown in Figure 4A and B, there was a significant increase in the serum MDA level in the CsA treatment group ($P = 0.01$). Coadministration of DMF reduced serum MDA to the level found in the control group ($P = 0.0003$ vs CsA; $P = 0.74$ vs normal). The CsA administration also resulted in modest increases in the renal tissue MDA level ($P = 0.26$), which was markedly ameliorated by DMF administration ($P = 0.001$ vs CsA).

Effects of CsA and DMF on Renal MPO Activity

As shown in Figure 4C, there was a significant increase in the renal MPO level in the CsA treatment group ($P = 0.03$). Coadministration of DMF significantly decreased the CsA-induced elevation of the renal tissue MPO level ($P = 0.03$).

Effect of DMF on Nuclear Factor Erythroid2-Related Factor1 Protein Expression in Renal Tissue

Renal tissue was evaluated via immunoblotting for protein expression of Nuclear Factor Erythroid2-Related Factor1 (Nrf2) in nuclear fractions (i.e., activated Nrf2) after 15 or 30 mg/kg administration of DMF. Dimethyl fumarate significantly increased the nuclear Nrf2 content in a dose-dependent manner (Figure 5A).

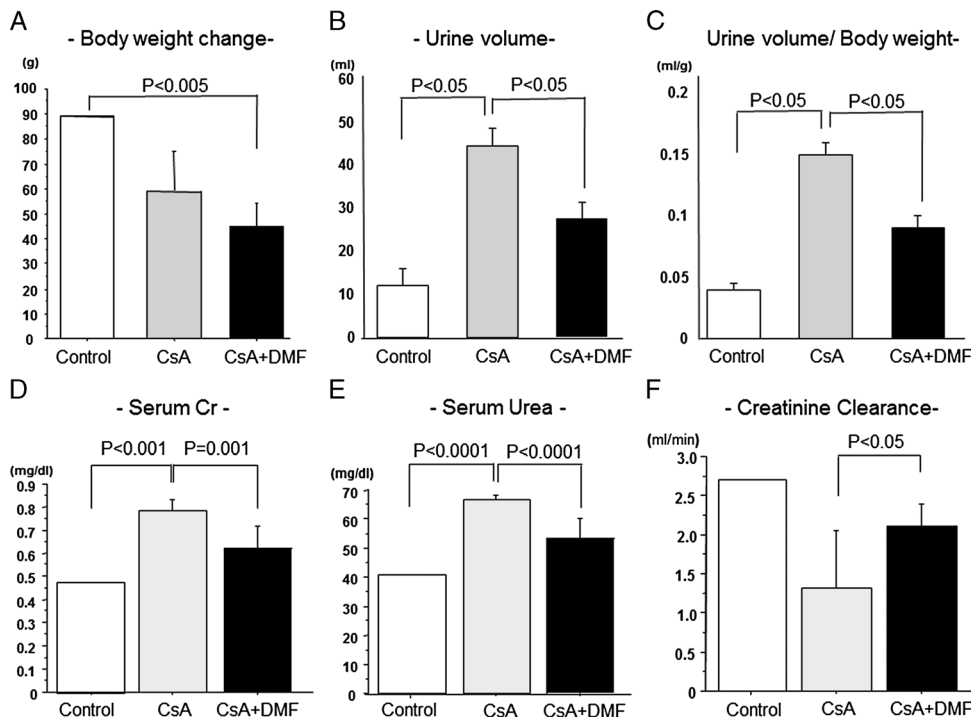


FIGURE 1. Effects of CsA and DMF on body weight, urine volume and renal function. Effects of CsA and DMF on body weight (A), urine volume (B), urine/weight (C), serum creatinine (D), serum urea (E), and creatinine clearance (F). Data represent mean \pm SD.

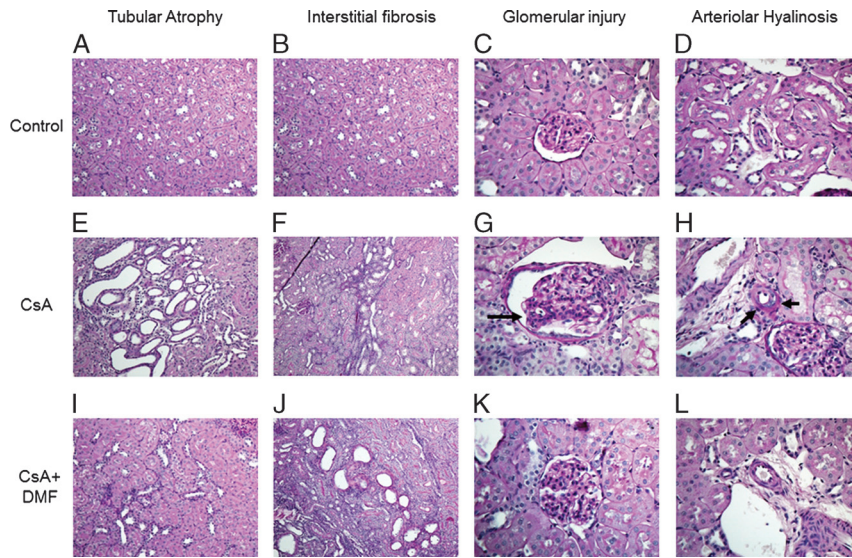


FIGURE 2. Histopathological changes. Kidney sections from Normal (A-D), CsA (E-H), CsA + DMF (I-L) groups. CsA-treated group showed histological injury included tubular cell vacuolization, tubular atrophy, interstitial fibrosis, glomerular mesangial matrix expansion, and arteriolar hyalinosis. These structural alterations were attenuated when CsA treatment was accompanied by DMF.

Effect of CsA and DMF on the Activities of Antioxidant Enzymes and NO System in Renal Tissue

Figure 5B shows the protein expression of antioxidant enzymes and NO synthase isoforms in the renal tissue. The CsA treatment resulted in a decrease in the expression of NQO-1 which was improved in CsA + DMF group ($P = 0.03$ vs CsA). The protein expressions of superoxide dismutase, catalase, and GCLC were not significantly different among the 3 groups.

The CsA treatment resulted in no difference in eNOS expression of ($P = 0.16$) and increase in iNOS expression

($P = 0.12$). Administration of DMF increased eNOS expression ($P = 0.18$) and decreased iNOS expression ($P = 0.15$).

DISCUSSION

In the present study, we have demonstrated that DMF may have a protective effect against CsA nephrotoxicity. We found that DMF maintained renal function and conferred protection against CsA nephrotoxicity. This was associated with and possibly, in part, mediated by attenuating oxidative stress (as evidenced by the reduction of lipid peroxidation product, MDA, and maintenance of the antioxidant

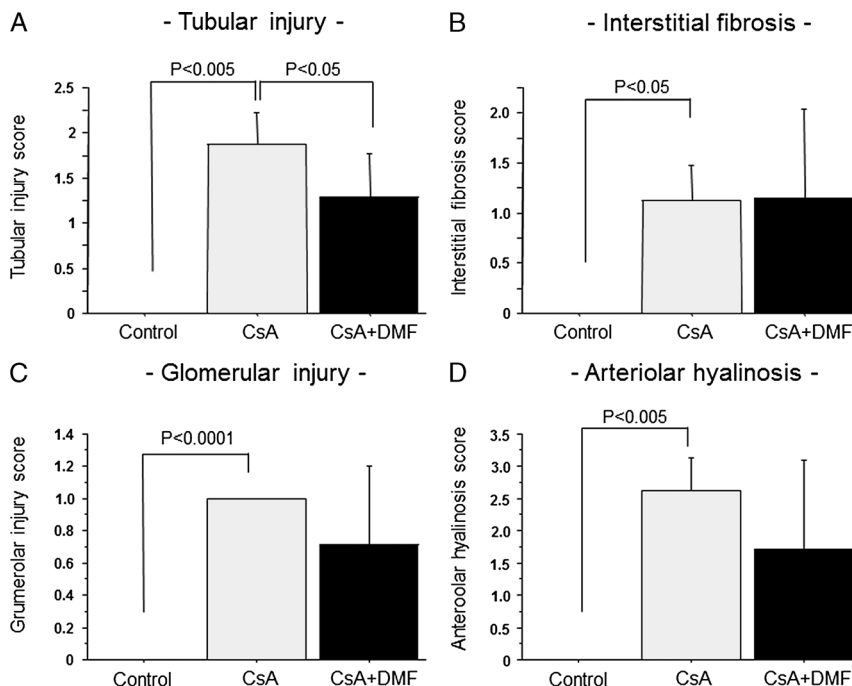


FIGURE 3. Histopathological scores. Effects of CsA and DMF on tubular injury (A), interstitial fibrosis (B), glomerular injury (C), and arteriolar hyalinosis (D). Data represent mean ± SD.

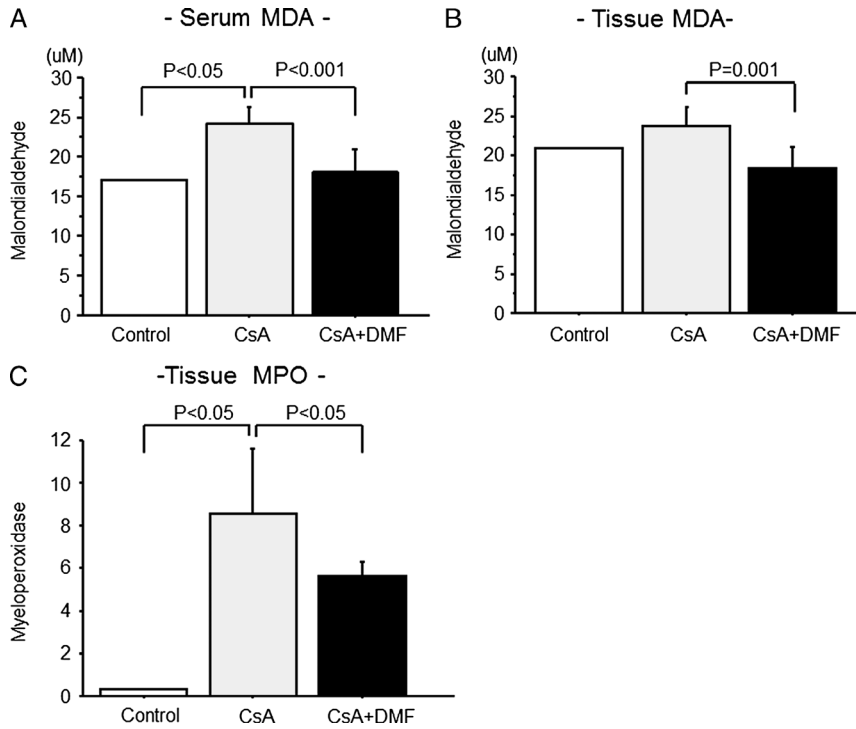


FIGURE 4. Effects of CsA and DMF on serum and tissue MDA, MPO activity. Effects of CsA and DMF on serum (A) and kidney (B) MDA (C) MPO activity. Data represent mean \pm SD.

enzymes) and inflammation (as evidenced by reduction of tissue MPO level and inflammatory cell infiltration).

Significant increases in serum Cr and urea and decreases in creatinine clearances were found in CsA-treated rats. These changes were associated with a remarkable rise in urine flow,

pointing to CsA-induced renal tubular dysfunction which was confirmed by histological evidence of TI, IF, GI, and arteriolar hyalinosis. These observations are consistent with earlier reports,^{18,19} which demonstrated significant changes in these renal functional parameters in both patients and

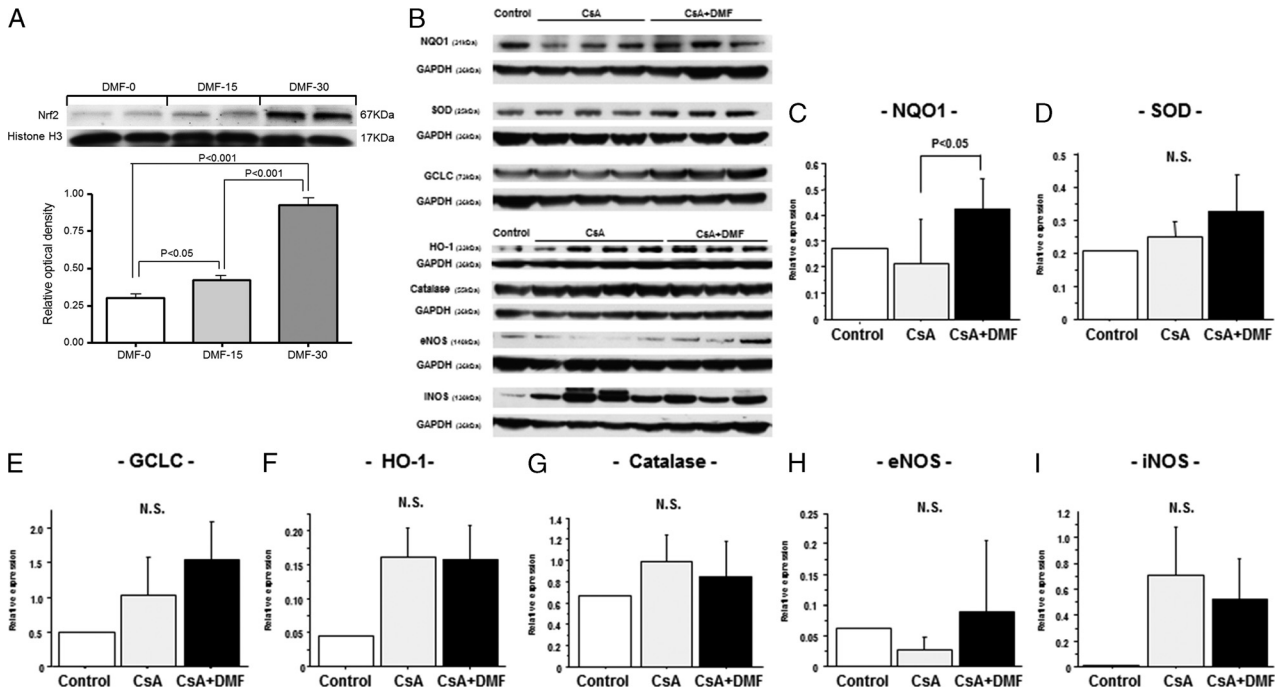


FIGURE 5. A, Effect of DMF on nuclear Nrf2 expression in kidney. Representative Western blots data are presented, depicting protein abundance of Nrf2 in the kidneys of Sprague–Dawley rats treated with vehicle, 15 or 30 mg/kg of DMF for 1 day. Histone H1 served as the loading control for Nrf2. Histone H1 served as the loading control for Nrf2 depicting protein abundance of Nrf2 in nucleus. B, Effects of CsA and DMF on anti-oxidant enzyme expressions in kidney. B, Representative Western blots of NQO-1, SOD, GCLC, HO-1, catalase, iNOS and eNOS. The bar graph summarizing the Western blot data of (C) NQO-1, (D) SOD, (E) GCLC, (F) HO-1, (G) catalase, (H) iNOS, and (I) eNOS. Data represent mean \pm SD.

experimental animals after CsA administration. The DMF administration reversed CSA-induced polyuria, suggesting its potential protective effect against TI.

The development of CsA nephrotoxicity in the present study was accompanied by the rise in renal and serum MDA, a byproduct of lipid peroxidation, suggesting excessive generation of reactive oxygen species and cellular damage. The increased levels of free radicals, lipid peroxidation products, and reduction of endogenous antioxidants after CsA administration have also been demonstrated in previous reports.^{6,20,21} Thus, we provided further support for the role of oxidative stress as a pathogenic factor in nephrotoxicity induced by CsA. Amelioration of renal dysfunction with DMF administration was accompanied by the reduction in serum and kidney tissue MDA levels which suggests that the salutary effect of DMF may be related to its antioxidant properties. This possibility has been substantiated by numerous reports demonstrating that DMF can activate the Nrf2–Kelch-like ECH Associated protein1 pathway^{10,11,14} and increase cellular levels of antioxidant enzymes, such as NQO1, HO-1, and GCLC. The Nrf2–Kelch-like ECH Associated protein1 signaling pathway plays a significant role in protecting the cells against various stresses including endogenous and exogenous oxidants, inflammatory stresses, and chronic exposures to cigarette smoke and other carcinogens.^{22–28} The cytoprotective effects of Nrf2 are mediated by transcriptional upregulation of genes encoding numerous antioxidant, detoxifying, and cytoprotective enzymes and related molecules.

In the present study, there were no significant changes in antioxidant enzyme expressions by CsA administration. Other studies indicate that HO-1 induction exerts renal protective effects in animal models including CsA-induced nephropathy, ischemia-induced acute kidney injury, radiation-induced nephropathy, and cisplatin nephrotoxicity.^{29,30} Therefore, this increase tendency may be explained by a self-defense system against CsA. The DMF administration significantly enhanced the NQO-1 protein expression. This was associated with the reduction of oxidative stress as evidenced by the fall in the serum and renal tissue MDA levels.

Via inhibition of nuclear translocation of NF- κ B which regulates expression of numerous genes encoding proinflammatory cytokines and chemokines, DMF has been shown to exert potent anti-inflammatory actions.³¹ Recent studies have also demonstrated that NF- κ B is involved in the regulation of iNOS expression.³² The induction of iNOS is also involved in CsA-induced renal damage.³³ Cyclosporine A has been shown to induce the expression of certain chemokines and adhesion molecules, such as monocyte chemoattractant protein-1, intercellular molecule-1, and vascular cell adhesion molecule-1, which activate or recruit the transmigration of inflammatory cells into the site of renal injury.³⁴ It is, therefore, conceivable that the protection afforded by DMF may be, in part, mediated through inhibition of NF- κ B activation and the associated inflammation. In fact, the renal tissue MPO activity (an indicator of neutrophil infiltration) which was significantly increased in CSA-treated rats was reduced by DMF administration pointing to its anti-inflammatory properties. Chronic CsA administration differentially affects NO synthase isoforms and NO production.³⁵ Cyclosporine A causes renal arteriolar vasoconstriction and increase renal

vascular resistance by decreasing eNOS-mediated NO production.³⁶ On the other hand, via induction of iNOS and consequent oxidative and nitrosative stress, CsA promotes renal damage.³³ Inducible nitric oxide synthase produces NO which reacts with superoxide generated by nicotinamide adenine dinucleotide phosphate-oxidase oxidases to form peroxynitrite. Previous reports showed CsA-induced significant decreases in eNOS expression while inducing significant increases in iNOS expression. We observed the same trend in the CsA-treated animals which was reversed with DMF. Our findings also suggest that the reactive oxygen species/NO systems play a role in the pathogenesis of CsA-induced renal injury and that DMF favorably influences these abnormalities.

Although antioxidant and anti-inflammatory activities may be the principal mechanism for the protection afforded by DMF, other unidentified actions cannot be excluded. Future works on this aspect are warranted.

Dimethyl fumarate has been widely used in Europe for the treatment of psoriasis vulgaris and psoriatic arthritis which are chronic inflammatory disorders, for over 20 years. Dimethyl fumarate has recently been investigated clinically in the United States for its neuroprotective effects. In a phase 3 trial for relapsing-remitting MS, oral DMF significantly reduced the proportion of patients who had a relapse, the rate of disease progression, the annual relapse rate, and the number of gadolinium enhancing lesions and new or enlarging T2 weighted hyperintense lesions on MRI.³⁷

The most common adverse reactions included flushing and GI events (nausea, diarrhea), which were classified as mild to moderate in nature. Most events occurred at the start of therapy and usually decreased over time. In clinical trials, 40% of patients experienced flushing. However, only 3% discontinued the drug because of adverse side effects. The most severe adverse reaction reported was a decrease in lymphocyte count. In clinical trials, mean lymphocyte count decreased by approximately 30% during the first year and then remained stable. Six percent of the patients experienced lymphocyte counts less than the lower limit of normal. However, the incidence and severity of infections was similar in the treatment and placebo groups. An increased incidence of elevated hepatic transaminases was observed in patients treated with Tecfidera (DMF clinical grade) compared to placebo, primarily in the first 6 months of treatment and most had levels that were less than 3 times the upper limit of normal. Discontinuation of the drug due to elevated hepatic enzymes was less than 1% and was similar for both the treatment and placebo patients. According to clinical trial for patients with MS, there is no report regarding the abnormal renal function. In this ongoing trial, patients have been taking more than 5 years.

We also checked islet cells, pancreas, liver, and intestine in rat for other projects after DMF treatment, showing no abnormality.

Furthermore, CSA and tacrolimus are calcineurin inhibitors and can both cause nephrotoxicity. It is therefore reasonable to assume that the mechanism by which they cause nephrotoxicity must be similar and that interventions that attenuate the adverse effects of one may be equally effective for the other. However, because the present study involved CSA alone, we cannot comment on the efficacy of DMF in the prevention of tacrolimus nephrotoxicity. Future studies are needed to address this important question.

In conclusion, the present study demonstrated that DMF treatment confers renal protection against CsA nephrotoxicity at least, in part, by attenuating oxidative stress and inflammation and enhancing or maintaining the antioxidant defense system. Our findings suggest that DMF may be a promising treatment option for limiting CsA-associated nephrotoxicity.

REFERENCES

- Halloran PF. Molecular mechanisms of new immunosuppressants. *Clin Transplant* 1996;10(1 Pt 2):118.
- Burdmann EA, Andoh TF, Yu L, Bennett WM. Cyclosporine nephrotoxicity. *Semin Nephrol* 2003;23(5):465.
- Tedesco D, Haragsim L. Cyclosporine: a review. *J Transplant* 2012;230386.
- Vaziri ND, Ni Z, Zhang YP, Ruzics EP, Maleki P, Ding Y. Depressed renal and vascular nitric oxide synthase expression in cyclosporine-induced hypertension. *Kidney Int* 1998;54(2):482.
- Parra Cid T, Conejo Garcia JR, Carballo Alvarez F, de Arriba G. Antioxidant nutrients protect against cyclosporine A nephrotoxicity. *Toxicology* 2003;189(1–2):99.
- Atessahin A, Ceribasi AO, Yilmaz S. Lycopene, a carotenoid, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rats. *Basic Clin Pharmacol Toxicol* 2007;100(6):372.
- Wongmekiat O, Leelarugrayub N, Thamprasert K. Beneficial effect of shallot (*Allium ascalonicum* L.) extract on cyclosporine nephrotoxicity in rats. *Food and chemical toxicology: an international journal published for the British Industrial Biological Research Association* 2008;46(5):1844.
- Schilling S, Goelz S, Linker R, Luehder F, Gold R. Fumaric acid esters are effective in chronic experimental autoimmune encephalomyelitis and suppress macrophage infiltration. *Clin Experiment Immunol* 2006;145(1):101.
- Ashrafian H, Czubik G, Bellahcene M, et al. Fumarate is cardioprotective via activation of the Nrf2 antioxidant pathway. *Cell Metab* 2012;15(3):361.
- Ghoreschi K, Bruck J, Kellerer C, et al. Fumarates improve psoriasis and multiple sclerosis by inducing type II dendritic cells. *J Exp Med* 2011;208(11):2291.
- Linker RA, Lee DH, Ryan S, et al. Fumaric acid esters exert neuroprotective effects in neuroinflammation via activation of the Nrf2 antioxidant pathway. *Brain* 2011;134(Pt 3):678.
- Meili-Butz S, Niemann T, Faslser-Kan E, et al. Dimethyl fumarate, a small molecule drug for psoriasis, inhibits nuclear factor-kappaB and reduces myocardial infarct size in rats. *Eur J Pharmacol* 2008;586(1–3):251.
- Onai Y, Suzuki J, Kakuta T, et al. Inhibition of IκB phosphorylation in cardiomyocytes attenuates myocardial ischemia/reperfusion injury. *Cardiovasc Res* 2004;63(1):51.
- Scannevin RH, Chollate S, Jung MY, et al. Fumarates promote cytoprotection of central nervous system cells against oxidative stress via the nuclear factor (erythroid-derived 2)-like 2 pathway. *J Pharmacol Exp Ther* 2012;341(1):274.
- Erman A, Chen-Gal B, Rosenfeld J. The role of eicosanoids in cyclosporine nephrotoxicity in the rat. *Biochem Pharmacol* 1989;38(13):2153.
- Erman A, Chen-Gal B, Zabudowski J, Rosenfeld JB. Cyclosporin A treatment enhances angiotensin converting enzyme activity in lung and serum of rats. *J Pharm Pharmacol* 1990;42(7):525.
- Racusen LC, Solez K, Colvin RB, et al. The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999;55(2):713.
- Chung BH, Li C, Sun BK, et al. Rosiglitazone protects against cyclosporine-induced pancreatic and renal injury in rats. *Am J Transplant* 2005;5(8):1856.
- Wongmekiat O, Thamprasert K. Investigating the protective effects of aged garlic extract on cyclosporin-induced nephrotoxicity in rats. *Fundam Clin Pharmacol* 2005;19(5):555.
- Amudha G, Josephine A, Varalakshmi P. Role of lipoic acid in reducing the oxidative stress induced by cyclosporine A. *Clin Chim Acta* 2006;372(1–2):134.
- Wongmekiat O, Gomonchareonsiri S, Thamprasert K. Caffeic acid phenethyl ester protects against oxidative stress-related renal dysfunction in rats treated with cyclosporin A. *Fundam Clin Pharmacol* 2011;25(5):619.
- Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. *Antioxid Redox Signal* 2005;7(3–4):385.
- Giudice A, Montella M. Activation of the Nrf2-ARE signaling pathway: a promising strategy in cancer prevention. *Bioessays* 2006;28(2):169–181.
- Surh YJ, Kundu JK, Na HK. Nrf2 as a master redox switch in turning on the cellular signaling involved in the induction of cytoprotective genes by some chemopreventive phytochemicals. *Planta Med* 2008;74(13):1526.
- Copple IM, Goldring CE, Kitteringham NR, Park BK. The Nrf2-Keap1 defence pathway: role in protection against drug-induced toxicity. *Toxicology* 2008;246(1):24.
- Li W, Kong AN. Molecular mechanisms of Nrf2-mediated antioxidant response. *Mol Carcinog* 2009;48(2):91–104. PMID: 2631094.
- Giudice A, Arra C, Turco MC. Review of molecular mechanisms involved in the activation of the Nrf2-ARE signaling pathway by chemopreventive agents. *Methods Mol Biol* 2010;647:37.
- Kundu JK, Surh YJ. Nrf2-Keap1 signaling as a potential target for chemoprevention of inflammation-associated carcinogenesis. *Pharm Res* 2010;27(6):999.
- Shin DH, Park HM, Jung KA, et al. The NRF2-heme oxygenase-1 system modulates cyclosporin A-induced epithelial-mesenchymal transition and renal fibrosis. *Free Radic Biol Med* 2010;48(8):1051.
- Abraham NG, Kappas A. Pharmacological and clinical aspects of heme oxygenase. *Pharmacol Rev* 2008;60(1):79.
- Hafez T, Moussa M, Nesim I, Baligh N, Davidson B, Abdul-Hadi A. The effect of intraportal prostaglandin E1 on adhesion molecule expression, inflammatory modulator function, and histology in canine hepatic ischemia/reperfusion injury. *J Surg Res* 2007;138(1):88.
- Dogan S, Aslan M. Hepatic ischemia-reperfusion injury and therapeutic strategies to alleviate cellular damage. *Hepato Res* 2011;41(2):103.
- Buffoli B, Pechanova O, Kojsova S, et al. Proximal prevents CsA-induced nephrotoxicity by reducing reactive oxygen species, iNOS, and NF-κB expression. *J Histochem Cytochem* 2005;53(12):1459.
- Park JW, Bae EH, Kim IJ, et al. Paricalcitol attenuates cyclosporine-induced kidney injury in rats. *Kidney Int* 2010;77(12):1076.
- Bobadilla NA, Gamba G, Tapia E, et al. Role of NO in cyclosporin nephrotoxicity: effects of chronic NO inhibition and NO synthases gene expression. *Am J Physiol* 1998;274(4 Pt 2):F791.
- Kou R, Greif D, Michel T. Dephosphorylation of endothelial nitric-oxide synthase by vascular endothelial growth factor. Implications for the vascular responses to cyclosporin A. *J Biol Chem* 2002;277(33):29669.
- Gold R, Kappos L, Arnold DL, et al. Placebo-controlled phase 3 study of oral BG-12 for relapsing multiple sclerosis. *N Engl J Med* 2012;367(12):1098.